Parasitology Research
https://doi.org/10.1007/s00436-021-07407-1

PROTOZOOLOGY - SHORT COMMUNICATION

Prevalence and diversity of haemosporidians in a migratory high-elevation hummingbird in North America

Adrienne M. Mackenzie[1] · Megan Dudenhoeffer[1] · Berit Bangoura[2] · Ravinder N. M. Sehgal[3] · Lisa A. Tell[4] · Braden L. Godwin[1] · Holly B. Ernest[1]

Received: 2 September 2021 / Accepted: 9 December 2021

Abstract

Hummingbirds (Trochilidae) are sensitive to environmental changes because of their extraordinary ecology, metabolism, and the highest red blood cell counts found in any vertebrate. These physiological attributes may render hummingbirds particularly susceptible to the effects of haemosporidian (blood parasite) infections. Much of the research on haemosporidians in hummingbirds has been conducted in South America; less is known about haemosporidian diversity and prevalence in North America. We sought to determine the prevalence and diversity of haemosporidians in a high-elevation species, the Broad-tailed Hummingbird (*Selasphorus platycercus*). Blood samples ($N=314$) from 25 sites in Colorado and Wyoming were screened for haemosporidians using microscopy ($n=311$) and PCR ($n=301$). Both microscopy and sequencing diagnostic techniques detected haemosporidians in the same 5 hummingbirds, with an overall prevalence of 1.59%. Positive samples were sequenced at the cytochrome *b* gene and identified *Haemoproteus archilochus* and two *Haemoproteus* sp. not previously detected in North America. No parasites of the genera *Plasmodium* or *Leucocytozoon* were detected. Our study provides the first report of the prevalence and diversity of haemosporidians in Broad-tailed Hummingbirds in the Rocky Mountains.

Keywords Avian · Broad-tailed hummingbird · *Haemoproteus* · Haemosporidians · Prevalence · Rocky Mountains · North American

Introduction

Hummingbirds (family Trochilidae) perform critical ecosystem services across their ranges in the Americas, such as pollination, and have the highest metabolism and red blood cell levels found in any vertebrate (Semmens et al. 2011; Moens et al. 2016). Because of their unique physiology, hummingbirds are particularly sensitive to the effects of environmental stressors, such as climate change, making them an important indicator of ecosystem health (Godoy et al. 2014). Several genera of haemosporidian blood parasites including *Leucocytozoon, Haemoproteus*, and *Plasmodium* are known to infect hummingbirds (e.g. Godoy et al. 2014; Bradshaw et al. 2017). High prevalence and diversity of haemosporidians has been reported in South American hummingbirds (Harrigan et al. 2014; Abad et al. 2021). Nevertheless, research concerning haemosporidians is extremely limited in North American hummingbirds, with only a few studies documenting the prevalence and diversity of haemosporidians and specifically in Californian hummingbirds (Godoy et al. 2014; Bradshaw et al. 2017; Galvin et al. 2021).

Broad-tailed Hummingbirds (*Selasphorus platycercus*) migrate annually in spring from Mexico to their summer breeding grounds in the western United States where they are the primary pollinators of many Rocky Mountain flowering plants (Calder et al. 1983; McKinney et al. 2012).

Handling Editor: Una Ryan

Adrienne M. Mackenzie and Megan Dudenhoeffer contributed equally

✉ Holly B. Ernest
Holly.Ernest@uwyo.edu

[1] Department of Veterinary Sciences, Wildlife Genomics and Disease Ecology Laboratory, University of Wyoming, Laramie, WY, USA

[2] Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA

[3] Department of Biology, San Francisco State University, San Francisco, CA, USA

[4] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA

Published online: 20 January 2022

Broad-tailed Hummingbirds exhibit high site fidelity and are therefore sensitive to environmental change such as changes in the phenology of flowering plants and potential mismatch between timing of migration and plant flowering dates (Calder et al. 1983; McKinney et al. 2012).

Our main objectives were to determine the prevalence of haemosporidians in Rocky Mountain Broad-tailed Hummingbirds, at their high-elevation summer breeding range in the Rocky Mountains, and to catalogue the diversity of detected haemosporidians. We additionally determined the phylogenetic relationship of detected haemosporidians to known hummingbird haemosporidians.

Materials and methods

Blood samples were collected from live hummingbirds in the field for this study and other research following procedures described in Godwin et al. (2020), during the summer breeding season from May–August 2015 to 2018 at 22 sites in Wyoming and Colorado (Fig. 1, Esri 2021). Approximately ~ 5–20 µl, (< 1% body weight) of blood was collected by clipping < 0.5 mm of a distal end of a toenail. Blood for microscopy was thin-smeared onto glass slides (Fisher Scientific ®, Pittsburgh, PA), and blood for DNA extraction was collected on Whatman FTA® preservation paper (GE Healthcare UK Limited, Buckinghamshire, UK), Nobuto sampling strips (Advantec®, Toyo Roshi, Ltd., Japan), or into EDTA capillary tubes that were then placed in 1.5 mL centrifuge tubes filled with 100% ethanol for storage. Federal Bird Banding Laboratory Permit #23,765, state (Wyoming Chapter 33 Permit #966 and Colorado Permits #17TRb2082 and #18TRb2082), and University of Wyoming Animal Care and Use Protocol Permit #20150716HE00183 were secured and animal care and use protocols were followed during animal handling and sample collection.

Blood smears on slides were fixed via step one alcohol dip Fisherbrand® Hema 3 Stat Pack (Fisher Scientific®, Pittsburgh, PA, USA) directly after collection on site for storage. In the laboratory, slides were stained according to the Wright-Giemsa staining protocol using the remainder of steps in the Fisherbrand® Hema 3 Stat Pack. The slides were mounted using mounting medium (Thermo Scientific® Cytoseal 60, Thermo Scientific® Kalamazoo, MI, USA) and glass cover slips (Fisher Scientific®, Pittsburgh, PA, USA). Mounted blood smears were examined completely using a light microscope and screened for haemosporidians stages at low magnification (× 400), and diagnostic interpretation was performed at high magnification (× 1000, oil immersion lens). Parasite identification was performed based on previous morphological descriptions of avian haemosporidians (Valkiūnas 2005; Matta et al. 2014; Valkiūnas and Iezhova 2018).

Blood samples stored on paper or in capillary tubes were extracted with Qiagen DNeasy® Blood and Tissue Extraction kits (Qiagen, Inc., Valencia, CA, USA) using modified protocols for tissues and blood. Blood stored on Whatman cards or Nobuto strips were hole-punched out, while the capillary blood tubes were ethanol-evaporated; all three sample types were placed into extraction tubes for digestion. To identify the presence of the genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* we used two nested-PCRs to amplify the cytochrome b gene of mitochondrial DNA following the methods of Hellgren et al. (2004). To detect *Haemoproteus* and *Plasmodium* sp. the first PCR contained the primer pair HaemNF and HaemNR2 and the second PCR contained HaemF and HaemR2 (Hellgren et al. 2004). To detect *Leucocytozoon* another nested PCR was used with the first PCR containing the primer pair HaemNFI and HaemNR3 and the second PCR: HaemFL and HaemR2L (Hellgren et al. 2004). Samples were submitted for bidirectional sequencing to Functional Biosciences Inc. (Madison, WI). Samples were replicated and sequenced 3 times to confirm their identify (Hellgren et al. 2004). Sequences were edited and aligned using the program Geneious Prime® 2021.1.1 (http://www.geneious.com. geneious.com). We used the Basic Local Alignment Search Tool algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) and MalAvi Database to determine the closest match to our sequences, requiring a 100% base pair match (identity) across the 478 bp region (Altschul et al. 1990; Bensch et al. 2009). We constructed a phylogenetic tree using the cytochrome *b* (cyt b) sequences (478 bp) of *Haemoproteus* from our study and 17 other *Haemoproteus* sequences downloaded from GenBank (all MalAvi lineage codes and GenBank accession numbers are available in SI 1). We specifically chose four *Haemoproteus* lineages from hummingbird hosts: *H. archilochus* (MalAvi HUMHA4, GenBank #KY560447), *H. witti* (MalAvi AM1, GenBank #KU364540), *H.* sp. (MalAvi 11PMALO, GenBank #KJ661248), and *H.* sp. (MalAvi LAMAME01, GenBank #MZ683462). The phylogeny was constructed using Bayesian analysis with MRBAYES v. 3.2 (Ronquist and Huelsenbeck 2003) using the general time-reversible model (GTR + I + T), tree selection followed Bradshaw et al. (2017), and with *Leucocytozoon quynzae* (from a hummingbird host) as the outgroup (MalAvi HELIAM01, GenBank #KF309188).

Results and discussion

We observed no abnormalities or signs of illness in any of the 314 Broad-tailed Hummingbirds examined. No hummingbirds were recaptured across years. Microscopy and PCR detected the same five *Haemoproteus* positive samples.

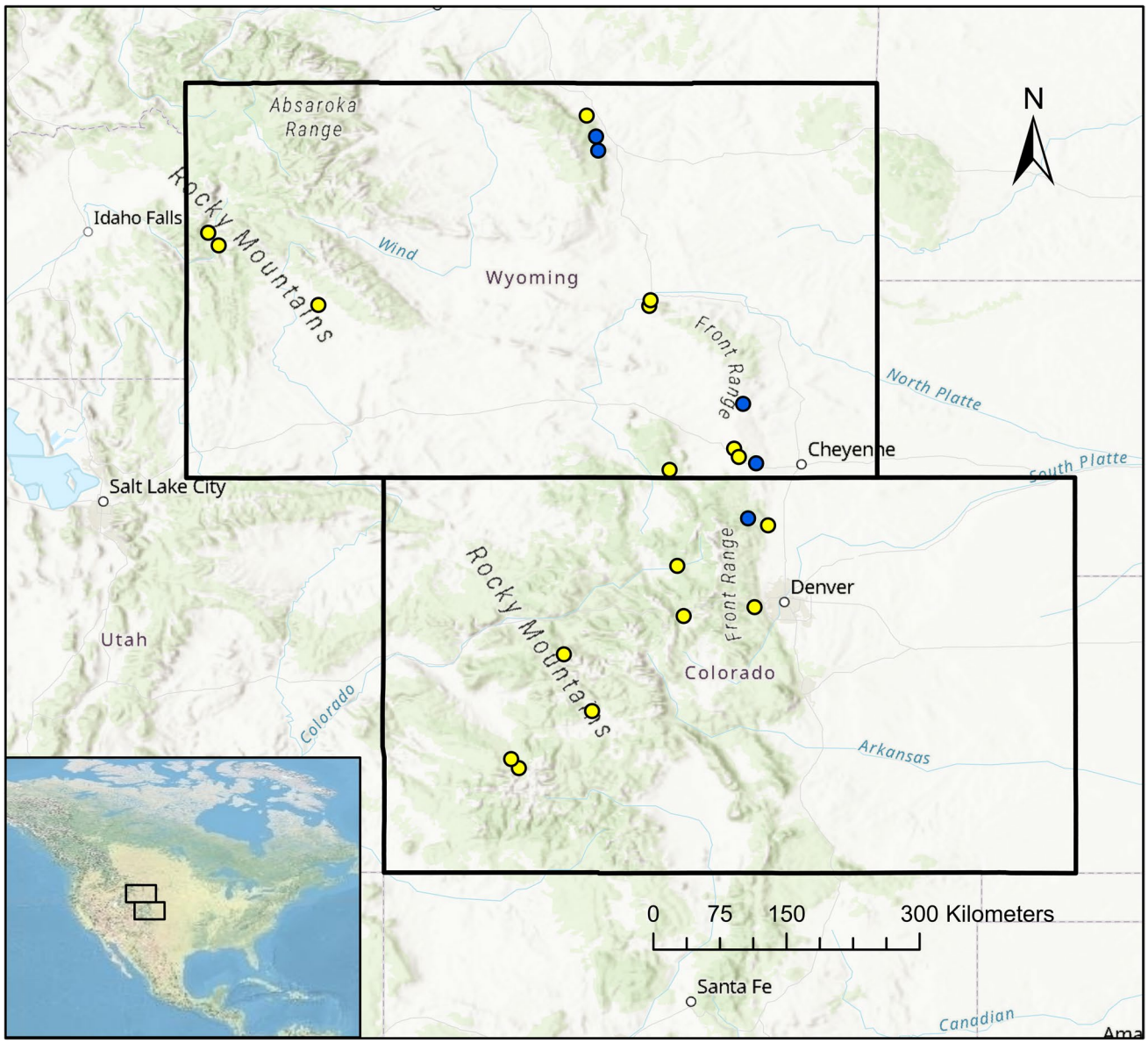

Fig. 1 Map of hummingbird sampling locations in Wyoming ($n = 13$) and Colorado ($n = 9$). Sampling locations where haemosporidian positive Broad-tailed Hummingbirds were detected are indicated in blue and locations where no haemosporidians were detected are in yellow. The map was created using ArcGIS Pro (version 2.8.0)

No *Plasmodium* spp. or *Leucocytozoon* spp. were detected using either method. All positive *Haemoproteus* positive hummingbirds were adults (at least one year old); two were male and three were female. Three positive hummingbirds were captured during June-July of 2016 and two during June 2018 and positive birds were each found at different sites (see Fig. 1, Esri 2021), four in Wyoming and one in Colorado. *Haemoproteus* prevalence was 1.61% by microscopy alone ($n = 311$), 1.66% by molecular methods alone ($n = 301$), and 1.59% for both methods overall ($n = 314$). In our study, the use of two methods provided solid evidence that haemosporidian prevalence is low in this Rocky Mountain population of Broad-tailed Hummingbirds. PCR is more sensitive than slide examination and may detect haemosporidians that undergo abortive development rather than successfully infecting the would-be host (Valkiūnas et al. 2014, 2016). Multiple gametocyte stages of *Haemoproteus spp*. were detected in erythrocytes using microscopy, suggesting that the positive samples we detected using PCR were truly infecting the hummingbirds hosts and were not due to abortive development (see SI 2.; Valkiūnas et al. 2014). Our results emphasize the importance and suitability of using blood smears alongside PCR results to confirm the presence and infection status of haemosporidians (Valkiūnas et al. 2014).

Our finding of low prevalence is similar to that reported for Anna's Hummingbirds (*Calypte anna*) in California: PCR prevalence of 1.27%; however, a microscopy prevalence of 4.76%, but lower than Black-chinned Hummingbirds (*Archilochus alexandri*) which were reported to have PCR prevalence of 10.58% and microscopy prevalence of 37.84% (Bradshaw et al. 2017). In reports for South American hummingbirds, haemosporidian prevalence is much higher, with some studies reporting a prevalence as high as 96% (Moens et al. 2016; Harrigan et al. 2014; Abad et al. 2021). The lower prevalence of haemosporidians in North American hummingbirds may be attributed to differences in biogeographic factors (temperate vs tropical environments), vector biology, avian species composition, or sampling effort (as previously, less research has been conducted in North America for hummingbird haemosporidians) (Godoy et al. 2014).

Molecular analysis revealed three distinct *Haemoproteus* lineages from the five positive samples. In one bird, we identified a lineage of the morphospecies *Haemoproteus archilochus,* that matched (100% identity) a sequence from an Anna's Hummingbird (MalAvi HUMHA4, GenBank #KY560447) in California, USA (Bradshaw et al. 2017). The *H. archilochus* species has so far only been detected in hummingbirds (Godoy et al 2014; Bradshaw et al. 2017) and is one of three *Haemoproteus* species known to infect hummingbirds, besides *H. trochili* and *H. witti* (Moens et al. 2016; Bradshaw et al. 2017). In 3 birds we detected a *Haemoproteus sp.* that matched a sequence from an Amethyst-throated Hummingbird (*Lampornis amethystinus*; MalAvi LAMAME01, GenBank #MZ683462) from Central Veracruz, Mexico (Álvarez-Mendizábal et al. 2021). The other unique sequence we detected was found in a single bird and differed by only 2 base pairs from our other *Haemoproteus sp.* sequence (MalAvi SELPLA01, GenBank #OL542488). These two *Haemoproteus* lineages found in our study have not previously been detected in North America. When we constructed a phylogenetic tree (see SI 1.; Bradshaw et al. 2017), both *Haemoproteus* sp. sequences grouped into single clade with a *H. syrnii* sequence from Bulgaria (MalAvi OTUSCO05, GenBank #KJ451480). Hummingbirds may come into contact with a diverse assemblage of vectors and avian hosts in Mexico prior to their migration to North America; however, Broad-tailed Hummingbirds could also be exposed to haemosporidian infection during summer breeding in the Rocky Mountains (McKinney et al. 2012). Further research on hemoparasite diversity in North America will help determine where birds contract haemosporidians and which vectors are responsible.

Neither *Plasmodium* spp. or *Leucocytozoon* spp. have been detected in North American hummingbirds (Bradshaw et al. 2017) however, Rodriguez et al. (2021) detected *Plasmodium spp.* in 11 non-hummingbird avian species in the Rocky Mountains, including a few resident bird species and pre-migratory juveniles. Thus, the vectors necessary to transmit *Plasmodium* spp. may be present in the Rocky Mountain region, along migratory routes, or in wintering habitats of those birds. Rodriguez et al. (2021) also found that the prevalence of haemosporidian genera (*Plasmodium* and *Haemoproteus*) was as high as 59% in non-hummingbird avian species in the Rocky Mountains. Biting midge arthropods of the genus *Culicoides* are reported to transmit *Haemoproteus* sp. to other avian species and are the most likely vector responsible for the spread of *Haemoproteus* spp. among hummingbirds (Žiegyte et al. 2017). Hummingbirds are thought to employ vector avoidance techniques by moving quickly and erratically at sunrise and sunset when vectors are most active (Godoy et al. 2014).

Our research provides novel insights into the prevalence and diversity of haemosporidians of hummingbirds in the Rocky Mountains of Wyoming and Colorado. We found higher diversity of haemosporidians in North American hummingbirds than previously reported. Additionally, our study provides baseline information on the health of this high-elevation migratory hummingbird. Future research efforts are needed to understand the differences in prevalence and diversity between hummingbird species and other avian species, the diversity of North American hummingbird haemosporidians, and which arthropod vectors are involved.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-021-07407-1.

Acknowledgements Access to sampling sites and sampling permission was provided by generous landowners, Wyoming Game and Fish Department, and Colorado Department of Parks and Wildlife. Field assistance was provided by (G. Ernest-Hoar, B. Hoar, A. Ernest-Beck) and the USGS Bird Banding Laboratory. Supplies and assistance for blood smear evaluations were provided by the staff of Wyoming State Veterinary Laboratory. B. Hoar created the map. G. Valkiūnas provided guidance on interpreting results.

Author contribution AMM and HBE conceived the project. HBE and BLG collected the samples. AMM stained the blood smears and carried out microscopy analyses with input from BB, RNMS, and LAT. AMM and BLG performed DNA extractions; MD conducted PCR analysis. MD carried out the data analysis with guidance from RNMS, LAT, and HBE with assistance from AMM. AMM, MD, and HBE wrote the manuscript. All authors read, revised, and approved the final manuscript.

Funding Funding was provided by the Wyoming Research Scholarship Program; NASA Space Grant Consortium; The University of Wyoming Kelly Ornithology Grant; Meg and Bert Raynes Wildlife Fund; Lewis and Clark Fund for Exploration and Field Research; IDeA Networks of Biomedical Research Excellence (INBRE); the Riverbend Endowment in Wildlife-Livestock Health and Wyoming Excellence Chair funds.

Data availability The unique DNA sequence detected during the current study is available on GenBank (accession #OL542488) and MalAvi (Lineage code SELPLA01).

Code availability Not applicable

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Abad CS, Tellkamp MP, Amaro IR, Spencer LM (2021) Incidence of avian malaria in hummingbirds in humid premontane forests of Pichincha Province, Ecuador: a pilot study. Vet World 14:889–896. https://doi.org/10.14202/vetworld.2021.889-896

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Álvarez-Mendizábal P, Villalobos F, Rodríguez-Hernández K et al (2021) Metacommunity structure reveals that temperature affects the landscape compositional patterns of avian malaria and related haemosporidian parasites across elevations. Acta Oecol 113:103789. https://doi.org/10.1016/j.actao.2021.103789

Bensch S, Hellgren O, Perez-Tris J (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol Ecol Resour 9:1353–1358. https://doi.org/10.1111/j.1755-0998.2009.02692.x

Bradshaw AC, Tell LA, Ernest HB et al (2017) Detection and prevalence of *Haemoproteus archilochus* (Haemosporida, Haemoproteidae) in two species of California hummingbirds. Parasitol Res 116:1879–1885. https://doi.org/10.1007/s00436-017-5463-5

Calder WA, Waser NM, Hiebert SM, Inouye DW, Miller S (1983) Site-fidelity, longevity, and population dynamics of broad-tailed hummingbirds: a ten year study. Oecol 56:359–364. https://doi.org/10.1007/BF00379713

Esri Inc (2021) ArcGIS Pro (Version 2.8.0). Esri Inc. https://www.esri.com/en-us/arcgis/products/arcgispro/overview. Accessed 21 Nov 2021

Galvin AN, Bradshaw AC, Myers BM et al (2021) Low prevalence of haemosporidians in blood and tissue samples from hummingbirds. J Parasitol 107:794–798. https://doi.org/10.1645/20-168

Godoy LA, Tell LA, Ernest HB (2014) Hummingbird health: pathogens and disease conditions in the family Trochilidae. J Ornithol 155:1–12. https://doi.org/10.1007/s10336-013-0990-z

Godwin BL, LaCava MEF, Mendelsohn B et al (2020) Novel hybrid finds a peri-urban niche: Allen's Hmmingbirds in southern California. Conserv Genet 21:989–998. https://doi.org/10.1007/s10592-020-01303-4

Harrigan RJ, Sedano R, Chasar AC et al (2014) New host and lineage diversity of avian haemosporidia in the northern Andes. Evol Appl 7:799–811. https://doi.org/10.1111/eva.12176

Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J Parasitol 90:797–802. https://doi.org/10.1645/GE-184R1

Matta NE, Lotta IA, Valkiūnas G et al (2014) Description of *Leucocytozoon quynzae* sp. nov. (Haemosporida, Leucocytozoidae) from hummingbirds, with remarks on distribution and possible vectors of leucocytozoids in South America. Parasitol Res 113:457–468. https://doi.org/10.1007/s00436-013-3675-x

McKinney AM, CaraDonna PJ, Inouye DW et al (2012) Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources. Ecology 93:1987–1993. https://doi.org/10.1890/12-0255.1

Moens MAJ, Valkiūnas G, Paca A et al (2016) Parasite specialization in a unique habitat: hummingbirds as reservoirs of generalist blood parasites of Andean birds. J Anim Ecol 85:1234–1245. https://doi.org/10.1111/1365-2656.12550

Rodriguez MD, Doherty PF, Piaggio AJ, Huyvaert KP (2021) Sex and nest type influence avian blood parasite prevalence in a high-elevation bird community. Parasit Vectors 14:1–12. https://doi.org/10.1186/s13071-021-04612-w

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Semmens DJ, Diffendorfer JE, López-Hoffman L, Shapiro CD (2011) Accounting for the ecosystem services of migratory species: quantifying migration support and spatial subsidies. Ecol Econ 70:2236–2242. https://doi.org/10.1016/j.ecolecon.2011.07.002

Valkiūnas G (2005) Avian malaria parasites and other Haemosporidia. CRC, Boca Raton

Valkiūnas G, Palinauskas V, Ilgunas M et al (2014) Molecular characterization of five widespread avian haemosporidian parasites (Haemosporida), with perspectives on the PCR-based detection of haemosporidians in wildlife. Parasitol Res 113:2251–2263. https://doi.org/10.1007/s00436-014-3880-2

Valkiūnas G, Lezhova TA, Križanauskienė A, Palinauskas V, Sehgal RNM, Bensch S (2016) A comparative analysis of microscopy and PCR-based detection methods for blood parasites. Published by: Allen Press on behalf of The American Society of Parasitologists. Accessed 27 July 2020

Valkiūnas G, Iezhova TA (2018) Keys to the avian malaria parasites. Malar J 17:17–19. https://doi.org/10.1186/s12936-018-2359-5

Žiegyte R, Markovets MY, Bernotiene R et al (2017) The widespread biting midge *Culicoides impunctatus* (Ceratopogonidae) is susceptible to infection with numerous *Haemoproteus* (Haemoproteidae) species. Parasit Vectors 10:397. https://doi.org/10.1186/s13071-017-2317-z